UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
WASHINGTON UTILIZATION RESEARCH BRANCH
Washington

CONFERENCE OF THE EVAPORATED MILK ASSOCIATION ADMINISTRATIVE TECHNOLOGISTS WITH THE DAIRY PRODUCTS SECTION, WASHINGTON UTILIZATION RESEARCH BRANCH

Thursday, February 18, 1954

NOTES ON THE MEETING

Introduction

The meeting was held in Room 3709 South Building, USDA, beginning at 10 o'clock. The following were present:

Industry Representatives

Dr. E. H. Parfitt, Asst. Executive Secretary, Evaporated Milk Association, 228 N. LaSalle St., Chicago 1, Ill.
Mr. E. M. Barker, Rochester Dairy Cooperative, Rochester, Minn.
Dr. W. J. Corbett, Dean Milk Company, 1126 Kilburn Ave., Rockford, Ill.
Mr. J. F. Hale, The Borden Company, 600 N. Franklin St., Syracuse 4, N. Y.
Dr. G. H. Hartman, White House Milk Company, Inc., Manitowoc, Wisc.
Mr. P. L. Haymes, United Milk Products Company, Cleveland, Ohio. (Member of USDA Dairy Advisory Committee)
Mr. Burdet Heinemann, Producers Creamery Company, P. O. Box 1427, S. S. Station, Springfield, Mo.
Dr. E. A. Louder, Pet Milk Company, Greenville, Ill.
Dr. E. B. Oberg, Carnation Research Laboratories, 8015 Van Nuys Blvd., Van Nuys, Calif.
Mr. Ray Powers, The Borden Company, 350 Madison Ave., New York, N. Y.
Mr. A. A. Scott, The Nestle Company, Inc., 2 William St., White Plains, N. Y.
Mr. J. M. Trimble, Indiana Condensed Milk Company, Lebanon, Ind.,
Mr. W. F. Widdifield, Consolidated Badger Cooperative, Shawano, Wisc.

Department of Agriculture Representatives

Washington Utilization Research Branch

Dr. John R. Matchett, Chief of Branch
Mr. Harry W. von Loesecke, Assistant Chief of Branch
Mr. S. B. Detwiler, Jr., Technical Assistant to the Chief
Dr. George E. Holm, Head of Dairy Products Section (chairman of conference)
Mr. Earle O. Whittier, Assistant Head of Dairy Products Section
Dr. Raymond W. Bell, Dairy Products Section
Dr. Harold R. Curran, Dairy Products Section
Dr. T. Foster Ford, Dairy Products Section
Dr. George R. Greenbank, Dairy Products Section
Mr. Oscar S. Sager, Dairy Products Section
Mr. Paul D. Watson, Dairy Products Section

Eastern Utilization Research Branch

Dr. Thomas L. McMeekin, Head, Animal Proteins Section

As chairman of the conference, Dr. Holm explained that it had been arranged in concert with Dr. Parfitt, to provide an opportunity for members of the Evaporated Milk Association to obtain a first-hand picture of the current activities of the Dairy Products Section, to discuss research needs of the evaporated milk industry, and to make suggestions regarding the future course of the program.

Dr. Holm outlined the change in administration of the dairy products work as effected by the recent reorganization of the Department of Agriculture. The work is under the general supervision of the Agricultural Research Service (Dr. Byron T. Shaw, Administrator). Under ARS, Dr. G. E. Hilbert, Director of Utilization Research, directs the activities formerly contained in the Bureau of Agricultural and Industrial Chemistry, together with the work on utilization of meat and dairy products of the former Bureaus of Animal Industry and Dairy Industry. The former BAIC activities include the four Regional Research Laboratories (Southern, at New Orleans, La.; Western, at Albany, Calif.; Eastern, at Wyndmoor, Pa.; and Northern, at Peoria, Ill.) which now become Utilization Research Branches. In addition, a fifth (Washington) Utilization Research Branch has been set up, with Dr. John R. Matchett as Chief of Branch. This Branch contains the Dairy Products Section (Dr. George E. Holm, Head), formerly the Dairy Products Research Laboratories.

Dr. Matchett, in welcoming the industry representatives, pointed out the potential advantages of the reorganization as concerns milk products research. Under the old setup, research on food uses of milk was concentrated in the Bureau of Dairy Industry (specifically, the Dairy Products Research Laboratories), while research on nonfood uses was concentrated in the Bureau of Agricultural and Industrial Chemistry (specifically, the Eastern Regional Research Laboratory). Under the new organization, all milk research is assigned to the Utilization Research group; hence a much closer integration of the program will be possible.

Dr. Matchett announced that notes would be made of the proceedings of the conference and later supplied to those present.

Dr. Holm successively introduced Messrs. Ford, Curran, Bell, Sager, and Watson of the Dairy Products Section, who reported on aspects of the program having particular interest to the evaporated milk industry. Each report, which was followed by a discussion period, is abstracted below. In the afternoon, an inspection of the laboratories and pilot plant of the Dairy Products Section was followed by a discussion with Dr. Henry W. Marston, Research Coordinator in the Office of the Administrator, ARS, and Executive Secretary of the Dairy Advisory Committee.

Particle Size Measurements on Milk and Their Application

T. Foster Ford

Work on particle size measurements was begun a number of years ago as a result of observations of certain anomalies in the behavior of milk; for example, it was found that some evaporated milk could be stabilized with phosphates, but only in the case of milk produced at certain times of the year. It was felt that a start toward the explanation of such phenomena could be made through fundamental studies of the molecular and colloidal structure of the components of milk.

More specifically, the objective of the work has been the determination of changes in the size, shape, composition, and other properties of milk particles, and of changes in the liquid system, during treatment with such agents as heat, acid, rennet, and detergents, and by dilution. Much of the work has involved the development of new types of centrifuge and new techniques for centrifugal analysis. Thus far, these techniques have been applied chiefly to fresh untreated milk, although some work has been done on heated milk.

The casein complex exists in milk as particles which are very large by molecular standards, having diameters of several hundred Angstrom units, or just under the lower limit of visibility of the light microscope. (One Angstrom unit = 0.000,000,1 mm.) There is a clear implication in the literature that the particles vary continuously in size, and hence cannot be resolved. A really continuous range of sizes is mathematically impossible; but particles formed by aggregation of a very small unit might cover a sensibly continuous range. The present assumption in the literature is that the unit is indeed very small.

It is Dr. Ford's contention that the casein-complex unit is larger than has previously been thought to be the case; and that, by application of any of several new centrifuge techniques that he has developed, casein-complex particles can be fractionated stepwise, the literature notwithstanding. Before discussing the results which led to these conclusions, it is necessary to state, in modified form, Stoke's law for the rate of fall of small spherical particles through a viscous medium under the force of gravity or a centrifugal field:

$$V = \frac{\Delta x}{\Delta t} = \frac{2}{9} r^2 \frac{d_1 - d_2}{k} F,$$

where

V = velocity of falling particle;
x = distance particle travels;
t = time of this travel;
r = radius of particle;
d_1 = density of particle, and d_2 = density of medium;
η = viscosity of medium; and
F = centrifugal force applied.

For a unit centrifugal field,

$$\frac{\Delta x/\Delta t}{F} = \frac{2}{9} r^2 \frac{d_1 - d_2}{\eta} = s.$$

The term s is known as the sedimentation constant, which is characteristic of a given colloidal particle in a given medium. It is sedimentation constants that are measured by ultracentrifuge techniques, and from which apparent molecular weights can be calculated.

It should be noted that s is proportional to the square of the radius of a particle, and hence to the ~~two~~-thirds power of its volume. As an example, since $2^{2/3} = 1.587$, a particle of volume 2 will fall 1.587 times as fast as a particle of volume 1.

By one of Dr. Ford's analytical centrifuge methods, the casein complex was separated from several samples of fresh milk, and the following sedimentation constants were obtained: 166 (182); 281; 372; 463; 591; 781; and 1,150. Now, if the first fraction is assumed to have particles of unit volume v, and if its sedimentation constant is taken as 181 instead of the experimental 166 or 182, then particles of volume 2v, 3v, 4v, 6v, 9v, and 16v will have calculated sedimentation constants of 288, 376, 457, 597, 782, and 1,150, respectively. This close agreement between experimental and calculated values indicated that the value 181 corresponded to the unit casein-complex particle, the equivalent molecular weight being 33,000,000 and the particle diameter, 640 Angstrom units. These findings were published by the International Dairy Congress, but have not yet been accepted by American journals in view of the literature statements previously mentioned.

Later, by another and independent analytical ultracentrifuge technique which is more accurate than the first method, Dr. Ford obtained a series of casein-complex fractions having sedimentation constants of 114, 181-2, 289, 457 ± 10, 728, and 1,142, respectively. Again there is a multiple relationship between successive fractions; but in this case the calculated molecular weight of the unit fraction is 16,500,000, just half of the value previously found.

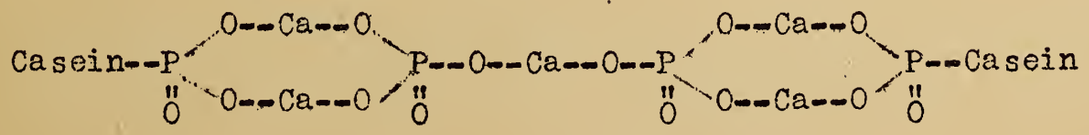
Preliminary results with a recently-developed optical centrifuge method, which involves photographing the boundaries between fractions of differing refractive index, have given sedimentation constants of 114, 181, 288, 457, and 1,150, as well as values for a number of intermediate fractions not found with the earlier techniques. For example, between 114 and 181, s values of

136 and 166 were obtained; and between 181 and 288, there were four new values. No fraction has been obtained whose sedimentation constant is less than 114; however, the greatest common divisor of all the fractions, on the basis of the two-thirds-power relationship, is 28.5, representing a molecular weight of 2,060,000. Further work is needed to determine whether this corresponds to a true unit casein-complex particle, or to a degradation product.

In these experiments, the relative proportions of particles of different size have changed continuously as a function of time. This fact has caused difficulties, especially in measurements by the original technique which necessarily extended over periods of several days, and where it was desired in each test to make use of samples from a single lot of milk.

By use of the analytical centrifuge techniques, studies have been made which bear on the chemical composition and structure of the casein complex. Portions of the same milk were centrifuged for increasing lengths of time, the casein complex being deposited as a jelly in progressively increasing amounts. Chemical analysis was made of the supernatant liquid from these samples. Plots of calcium content as a function of nitrogen content gave a straight-line relationship, indicating that the Ca/N ratio of the casein complex was the same over the whole range of particle sizes. On the other hand, plots of organic phosphorus as a function of nitrogen, while straight-lines over most of the range, fell off at the higher levels of nitrogen. At the same time the acid-soluble phosphorus tended to increase at the higher levels. These changes in the organic and inorganic phosphorus occur in the region representing the largest of the casein-complex particles, and comprising 5 to 15 percent of the total casein complex. The changes may indicate sorption of both inorganic phosphates and non-phosphorus nitrogenous materials; they may also indicate the existence of two kinds of casein in milk.

The chemical analyses permit some conclusions concerning the structure of the major fraction of the casein complex. It has the P/N ratio that it would have if the protein were composed of alpha-, beta-, and gamma- caseins in the proportions 16 to 4 to 1. The Ca/P ratio indicates that the unit casein-complex particle comprises two calcium caseinate molecules linked by one molecule of tricalcium phosphate, as follows:



As previously indicated, centrifuge studies on heated milk are still in the preliminary stages. It has been noted that heating produces distinct shifts in the particle size distribution and in the calcium distribution. There is an increase in the proportion of large particles, and also in the proportion of small particles, both at the expense of intermediate sizes. The small particles have a tendency to form jellies. The gel structure is such that, while it has strength, the larger particles can settle through it.

Discussion

Detergents

Dr. Holm: Asked about the effect of detergents on particle size. Dr. Ford: Some detergents will cause gelation, while others convert milk to a translucent substance in which the large particles have disappeared, and only a few very small ones remain.

Gelation

Dr. Oberg: Requested further discussion of gelation. Dr. Ford: Because gelation greatly affects the stability of milk in the can and hence is of prime importance to the evaporated milk industry, he is anxious to correlate his ultracentrifuge studies with viscosity measurements. He has at hand an accurate rotating-cylinder viscosimeter, and hopes to make use of it soon.

Dr. Ford, continuing: Jellies may be formed in at least two ways. We could start with small dispersed particles which, on heating would puff up like rice to form a jelly. This type of jelly would not be particularly elastic; fat and other large particles would not diffuse through it; and dilution would destroy the gel. A second type of jelly might be formed from a medium containing large globular particles which on heating would explode to form a brushlike structure with interlaced fingers, reminiscent of prickly burs, or octopi. This structure would have elasticity which would not easily be destroyed on dilution; other particles would be expected to pass through the brushwork structure. If we could discover how to make large particles small and small particles large, we could perhaps control the gelation.

Mr. Heinemann: When you speak of a casein-complex particle, would you say that it is a molecule? Dr. Ford: Would prefer not to commit himself on that.

Mr. Heinemann: Did you use fresh milk in your work? Dr. Ford: Yes, it was plain untreated milk. The age of the milk must be considered, since in a given series of centrifuge runs, samples from the same lot of milk must be used, and considerable time may elapse in a series of runs. In the original sedimentation experiments, mentioned previously, the age of the samples was a very important factor which made it necessary to plot data in three dimensions.

Dr. Parfitt: Requested further discussion of gelation as concerns high-temperature, short-time heating of milk as compared with heating at lower temperatures for longer periods of time. Dr. Ford: Chemical reactions take time, and the length of time might be expected to be greater, the larger the molecules or particles involved. Also, the phosphorus analyses indicate that there may be two chemically different kinds of casein complexes in milk, and these complexes might be expected to react differently to heat, and at different rates. These different rates, combined with the limitations of sizes, would affect the overall results of heating. Should expect that consideration of reaction kinetics would be important here.

Mr. _____: In some evaporated milk that had been overheated, he

found "octopi" solarge that they could be lifted out in strings. Dr. Ford: That is a rather large molecular structure. However, he has observed gelation by acid and rennet, through a microscope. The particles, which are originally in rapid motion, adhere by contact to form chains and nets of chains. Incidentally, bacteria have been observed in the process of being trapped in such networks. This shows considerable strength.

Continuation of Work

Dr. Parfitt: Dr. Ford's work will continue as indicated? Dr. Holm: Yes. He has just finished his studies on fresh milk, and has made a beginning on evaporated milk. There are many problems ahead. Dr. Parfitt: Dr. Ford is to be complimented on getting his analytical methods worked out.

Personnel

Dr. Corbett: Asked about the number of Dr. Ford's assistants. Dr. Holm: Dr. Ford has three assistants and is trying to obtain another.

The Influence of Iron and Manganese on Bacterial Spore Formation in Milk

Harold R. Curran

In the course of a series of investigations of the behavior of bacterial spores in milk, the Dairy Products Section has made some significant observations concerning the influence of metals, specifically iron and manganese, on spore formation. This study, begun last year, has shown that salts of these metals, while not affecting spore germination, do markedly transform the spore-generating potential of the medium, as regards certain species of bacteria. The work has practical implications with reference to the appearance of contaminating trace metals in milk during commercial handling and processing. It may lead to an explanation of the occasional sudden appearance in condensery milk of large numbers of spores which make sterilization difficult and uncertain.

The bacteria used in this work comprised various strains of 10 species of Bacillus, of which the results with B. subtilis will be here reported. The trace metals were chiefly iron and manganese, generally introduced as $FeCl_3 \cdot 6H_2O$ and $MnCl_2 \cdot 4H_2O$, in concentrations of metal ranging from 2 to 280 parts per million. Soluble salts of numerous other metals -- potassium, sodium, magnesium, calcium, cadmium, zinc, cobalt, nickel, strontium, tin, copper, and cerium -- also were tested; but these either reduced sporulation or had no significant influence, and will not be discussed further.

In the first part of the work, the substrate for the bacteria was autoclaved skim milk. For purposes of comparison, another series of experiments was conducted in which the substrate was one of the following common nutrient media: Plain broth (beef extract and peptone); yeast broth (yeast extract, peptone, and potassium monohydrogen orthophosphate); pork infusion (lean pork extract, peptone, tryptone, and potassium monohydrogen orthophosphate); and peptone broth (peptone only).

Sporulation in Milk

Skim milk containing iron or manganese in quantities from 0 to several hundred parts per million was seeded with spores of B. subtilis and incubated at 37°C., the p_H being unadjusted. At intervals from 0 to 120 hours, the samples were heated at 85°C. to kill vegetative growth, portions were plated on nutrient agar, and the plates were incubated for 48 hours and counted. Colonies developing on the plates were assumed to have originated from spores.

All samples showed a rapid initial germination of spores, as indicated by a decreased spore count at 12 hours. Thereafter, although vegetative reproduction was heavy in all cases, spore synthesis in the absence of added metals was negligible over a 120-hour period. In the presence of suitable concentrations of metal, however, spore synthesis up to 48 hours was rapid. In the case of added manganese, spore formation was in inverse proportion to the metal content, being very rapid at 2 ppm, and slightly less rapid at 20 and 200 ppm. In the case of added iron, the relationship was direct: At 2 ppm spore formation was negligible, at 20 ppm it was moderate, and at 200 ppm it was approximately as rapid as with 2 ppm of manganese. After 48 hours there was little further increase in sporulation. It is noteworthy that while manganese was effective in low concentration, a hundred times as much iron was required to produce a comparable result; this probably accounts for the fact that the spore-promoting activity of iron has been previously overlooked. Increasing the concentration of added iron to 400 and 600 ppm usually resulted in a further increase of the spore crop when the p_H, which had been made acid by the iron salt, was adjusted nearly to the neutral point.

Sporulation in broth media

In view of the results obtained with milk, it was of interest to ascertain if the spore-promoting function of iron and manganese could be similarly demonstrated in other complex organic media, particularly those used in the laboratory as substrates for the growth of sporogenic bacteria. A second set of experiments was therefore performed, in which B. subtilis was grown on plain broth, peptone broth, yeast broth, or pork infusion, with or without the addition of iron (200 ppm) or manganese (2 ppm). In all cases the p_H was adjusted to neutrality. The results showed that, as in the case of milk, vegetative reproduction was heavy in all samples; but again, the samples containing no metal produced few or no new spores, while those containing either iron or manganese produced a plentiful crop. As concerns the different substrates, the simpler plain broth and peptone broth caused generally better sporulation than did the more complex yeast broth and pork infusion.

Sporulation as Influenced by Form in Which Iron is Supplied

In the foregoing experiments, iron where used to fortify the medium was added as ferric chloride. An additional series of tests was made in which B. subtilis was cultured in milk, the fortifying agent being either ferric chloride, ferric thiocyanate, ferric oxide, or reduced iron (all at 200 ppm), or ferrous chloride (at 280 ppm). The highly-ionized ferric and ferrous chlorides, in the concentrations used, were equally effective in promoting heavy sporulation. The feebly-ionized ferric thiocyanate promoted moderately

heavy spore formation, while ferric oxide and reduced iron had no effect. The results obtained with the thiocyanate suggest that not only the ions, but also iron in the nonionic form, contribute to spore formation.

Sporulation as Influenced by Rust Contamination

The effects which extraneous iron may induce in sporogenic organisms in milk suggest that contamination with iron may play a role in certain problems of milk preservation; the wide distribution of this element in soil and the many routes by which it may enter milk lends substance to this possibility. Of special interest is the effect upon sporulation of rust contamination in milk handling equipment. To obtain some light on this point, a sample of sterile skim milk was treated with rust scraped from a used rusting dairy utensil, while a second portion from the same source served as control; the two samples were similarly inoculated with spores of B. subtilis, and plate counts were made at zero hours and after the usual periods of incubation. In a second experiment, equal volumes of well-mixed non-sterile fresh milk were stored at 0°C. for 24 hours in each of two similar sterile milk cans, one of which was rust-free, while the other contained on its inner surface large areas of rust. Aliquots of each sample were then incubated at 37°C., with and without previous inoculation with spores of B. subtilis. Spore counts at the usual periods provided the desired data.

In the first experiment, active sporulation was observed in the sample containing rust from the dairy utensil, the results being comparable to those observed previously with soluble iron salts. In the second experiment, the storage of milk in the rusty can actively promoted the formation of new spores, whereas storage in the rust-free container had no significant effect. It should be noted that in the case of the rusty container, iron effects were produced both in the sample to which B. subtilis spores had been added, and in the uninoculated sample; this indicates the presence of iron-responsive sporogenic bacteria in the natural milk flora.

Discussion

Dr. Parfitt: In the rusty can, how would the iron be transmitted to the milk? as the lactate? Dr. Curran: Probably so. Citrates or other soluble salts also might be involved. It takes only a few hours' standing of the milk to produce the effect.

Mr. Heinemann: Did you determine the concentration of iron in the milk that stood in the rusty can? Dr. Curran: No. We have seen published figures indicating that milk as it comes from the cow has an iron content of perhaps 2 ppm; but we know of no data on iron picked up from the container or extraneous sources. Mr. Heinemann: His point is that in the experiments first reported, it required from 20 to 200 ppm of iron to promote sporulation, whereas a concentration of 2 ppm had little influence. Dr. Curran: The results that I first mentioned, on spore formation in milk containing added iron salts, are readily reproducible. We can safely conclude, therefore, that the sample of milk to which rust had been added, had a soluble iron content of at least 50 ppm. Incidentally, there are certain non-spore-forming organisms in milk whose growth is stimulated by very small amounts of iron. An example is Aerogenes, a troublesome gas-former in milk.

Dr. Louder: Are there metals which will inhibit spore formation? Dr. Curran: In the present work we studied a variety of metals, of which iron and manganese promoted spore formation, while the remainder either reduced spore formation in the concentrations tested, or had no significant influence.

Dr. Louder: What is "menadione", which is being fed to stock? Dr. Curran: Understands this to be 2-methyl-1,4-naphthoquinone. Workers at Florida State University have claimed that when minor amounts of this substance are added to the feed of lactating cows, or directly to milk, souring is retarded. This work was discussed in a paper at the American Chemical Society meetings in the spring of 1953. The Dairy Products Section has done only a very minor amount of work on it, and hence we are reserving opinion on its effectiveness.

Dr. Parfitt: To return to the subject of iron, if iron were exposed in an evaporated milk can and the sterilization were insufficient to give a full kill, would the iron contribute to spoilage? Dr. Curran: Suspects that the iron in this case would have little effect. Iron does not promote the germination of spores, but rather their formation.

Dr. Parfitt: Is there any recent work on the reason why spores go into the vegetative stage? Dr. Curran: Sporulation occurs when nutritional or environmental conditions become unfavorable to growth; when these conditions again become favorable, most of the spores germinate promptly, and vegetative multiplication takes place. A very small minority of spores frequently exhibit delayed germination under favorable growth conditions; the causes of this phenomenon are as yet quite obscure.

Mr. Heinemann: If you took a sample of sterile milk, added iron to one portion, then added B. subtilis to both portions and incubated, which portion would have the highest cell count? Dr. Curran: There would be more cells in the sample containing iron. In the case of manganese, would say that the effect on cell growth would be negligible; the effect is entirely on the production of spores.

Mr. Heinemann: Percentagewise, do you have more spores in relation to the total population, in the case of iron? Dr. Curran: Yes.

Dr. Corbett: What is the effect of metals on thermal death times? Dr. Curran: There is little effect. Enterococcus does show a measureable increase in heat resistance when it is grown in the presence of iron, but this effect is only of the order of 2 or 3°C.

Preservation of Milk by Freezing

Raymond W. Bell

Frozen Concentrated Skim Milk for Use in Baking

The Dairy Products Section has been interested in frozen concentrated skim milk as a source of milk solids-not-fat in bread. In the course of experiments in this field, skim milk was divided into two portions, one of which was forewarmed at 150°F. for 30 minutes, the other at 180°F. for the same period. The former temperature corresponds roughly to that used in pasteurization, the latter to the heat treatment considered best when skim milk is to be dried and used in making bread.

After forewarming, each portion was concentrated to about 40 percent solids content, packaged in small cans, frozen, and stored at 0°F. for a year. Each month or two, samples were thawed, examined, and incorporated into bread doughs at the rate of 6 parts of skim solids to 100 parts of flour. Bread was made from the doughs, and evaluated by standard scoring procedures (flavor, loaf volume, crumb, color, etc.).

In the course of the year, the body of the concentrated skim milk deteriorated until the thawed samples were curdy, coarse, and even whey-ed off. Also, the flavor deteriorated, gradually becoming old, stale, and even oxidized. Yet this material continued to be as good a source of milk solids for baking as when the samples were fresh. Samples from milk forewarmed at 180°F. gave better results than did those where the forewarming temperature was only 150°F.

The foregoing work was described in the Journal of Dairy Science for July 1953.

More recently, frozen storage of concentrated skim milk has been compared to drying as a means of preserving milk solids-not-fat for use in bread. Satisfactory results were obtained in all baking tests. However, results with dried skim milk solids were slightly superior to those with concentrated skim milk.

Discussion

Dr. Parfitt: Were there any differences in the milk before and after freezing? Dr. Bell: No; the rate of freezing is a minor factor. It is the storage conditions -- time and temperature -- that are important.

Dr. Bell: It may be of interest to add that in some cases, gelation was obtained on freezing the samples. Also, deterioration was first evidenced after two or three weeks of storage; that was true, incidentally, of concentrated whole milk as well as of concentrated skim milk.

Dr. Oberg: In connection with deterioration, did you make use of antioxidants? Dr. Bell: Not in the work just reported. We have used antioxidants in other cases, however, and find that they are helpful.

Dr. Greenbank: Did the loaf volume remain the same in both sets of samples? Dr. Bell: There was a difference in favor of the high-heat-treated milk.

Refrigerated Storage of Evaporated Milk

Dr. Bell next discussed the advantages of storing evaporated milk under refrigeration. In this connection, he exhibited graphs taken from an article by B. H. Webb et al., of the Dairy Products Section, appearing in the November 1951 issue of the Journal of Dairy Science.

Evaporated milk in cans was stored in an undisturbed condition for two years at temperatures ranging from 30 to 100°F. Thereafter, the still undisturbed samples were quick-frozen, removed from the cans, and cut horizontally into three equal portions. Each portion was analyzed for fat and protein.

At temperatures of 50°F. and below there was no separation of fat or protein. At 60° there was appreciable separation, which increased rapidly at higher temperatures. Fat rose at a relatively fast rate into the upper fraction, whose fat concentration at 86° storage was twice that of the sample before storage. The rising fat carried with it considerable quantities of the protein. Much of the remaining protein settled toward the bottom. Thus, in the cans stored at 86°, the protein in the middle fraction was markedly depleted.

The stability of the samples stored at 50° and below is a reflection of increased viscosity at these temperatures.

Discussion

Dr. Bell: The advantages of refrigerated storage of evaporated milk have been demonstrated in the foregoing work. He has been informed of one disadvantage, which is that when the cans are removed from refrigeration, condensation of moisture causes rusting. He inquired whether this could not be prevented by coating the cans with shellac or other rust inhibitor.

Dr. Parfitt: The sweating of cans is a problem that industry has learned how to handle, and hence is not as serious as Dr. Bell's informant would indicate. The general procedure is to watch the dew point carefully. During the War, can coatings were tried as a camouflage measure, but no one is now using such coatings for rust prevention. Additionally, very little milk is now stored in "bright" cans, i.e., without a label.

Dr. Louder: Some firms store cans on pallets covered with an insulated wrapper. When the pallets are removed from refrigeration, any moisture will be condensed on the wrapper.

Dr. Louder: Protein separation is an important problem, which appears to be most acute in the case of Southern milk. Dr. Parfitt: Protein separation in Southern milk may have to do with processing methods, since the situation is gradually being improved.

Mr. Widdifield: Referring to the protein- and fat-separation curves on Dr. Bell's graphs, is there a critical temperature below which refrigeration has no advantages? Dr. Bell: Evidently 50° is the critical temperature, below which little further advantage is obtained.

Dr. Oberg: Asked about the viscosity of the samples stored at the different temperatures. Dr. Bell: This information is given in Webb's publication, cited above.

Dr. Parfitt: The work which Dr. Bell has described has already resulted in a trend toward cool storage on the part of evaporated milk producers.

Effect of Heating on Characteristics of Milk

Dr. Bell discussed one phase of a study on the effects of heat on milk, which it is hoped will aid in learning how to prepare a superior evaporated milk.

Samples of skim milk were held at temperatures of 150°, 168°, and on up to 300°F. in 18°-increments, for a period of 20 minutes. In all cases the preheating time and the cooling time were one minute each. The samples were then examined for pH, color, and the amount of non-volatile reactive sulfur that was formed during heating.

The results, which were displayed in a graph, showed a sharp increase in acidity at the higher temperatures, above 240°F. The color increased at temperatures above the boiling point, especially above 250°. The curve for non-volatile sulfur rose and fell in the region 170° to 230°F., then rose again at about 250°. Possibly the reason for the second rise is that, with the formation of amino acids from serum protein and of formic acid from the lactose, the sulfur groups were re-exposed.

None of these curves is ideal, in that none shows a linear relationship to the amount of heat put into the skim milk.

If skim milk of higher solids content had been used in this experiment, the heat effects would have occurred more rapidly and to a greater extent, so that the curves would have been steeper and their peaks higher.

Detergent Test for Fat in Milk and Milk Products

Oscar S. Sager

In the Babcock test for fat in dairy products, the use of sulfuric acid has long been recognized as expensive, corrosive, and hazardous to personnel. Efforts to develop a more satisfactory test have been made by various workers, including Schain (Science 110,121 (1949)), who proposed the use of two detergents -- one anionic, for dispersing proteins and releasing the fat, and the other nonionic, for extracting and raising the fat. Evaluation of the Schain test indicated that, while satisfactory from the standpoint of noncorrosiveness, it suffered from certain major defects which led to inconsistent results over the range from low-fat milk to high-fat milk. In consequence, the Dairy Products Section has developed a new method which employs a polyphosphate salt and a nonionic detergent as the principal reagents. This method, known as the "BDI Detergent Test for Butterfat in Milk and Other Dairy Products", was published in the Proceedings of the Milk Industry Foundation Convention (Chicago), September 24-26, 1952.

The present report will comprise an outline of the test as published in 1952, together with a discussion of recent improvements and the results of comparisons with the Babcock and Roese-Gottlieb methods.

BDI Detergent Test as Applied to Milk

Reagents

A. 7.0 g. sodium tetraphosphate and 3.0 g. Triton X-100 made up to 100 ml. with distilled water.

B. 50 percent methyl alcohol.

Procedure

The test bottle and milk pipet are the same as in the official Babcock test (AOAC Methods of Analysis, 7th Ed., 1950, pp. 233-234). Likewise, the centrifuge, calipers, and water bath for "tempering" the test, are the same. The milk is prepared as directed in the Methods, p. 227, which specify bringing the milk to 20°C. before sampling. The test is then conducted as follows:

Part A. With a pipet transfer 18 g. of prepared sample to milk test bottle. Blow out milk in pipet tip after free drainage has ceased. Add 5.0 ml. of reagent "A" portionwise so as to wash all traces of milk into bulb. Mix by shaking. Transfer bottle to bath of boiling water, level of water covering level of milk in bottle. After about 5 minutes in bath, shake to remix raised cream and replace in bath for 10 more minutes. Remove from bath without remixing contents.

Part B. Method using centrifuge. While bottle is still hot, add 50 percent methyl alcohol to top of graduated scale. (No water is added in this procedure.) Allow alcohol to run down side of neck. Transfer bottle to unheated centrifuge and rotate centrifuge for 2 minutes. Transfer bottle to water

bath maintained at 55-60°C., immerse it to level of top of fat column, and leave for at least 15 minutes, until column is in equilibrium and lower fat surface has assumed final form. Remove bottle from bath, wipe it, and with aid of dividers or calipers measure the fat column in terms of percent by weight, from lower surface to highest point of upper meniscus (bottom-to-top reading).

Part B. Alternate method not using centrifuge. While bottle is still hot, add 50 percent methyl alcohol, pouring it down side of neck, until contents of bottle reach into neck of bottle, but not above zero graduation on scale. Add water until contents reach top of calibrations. Transfer bottle to warm water bath at 55-60°C. for tempering, and continue as in Part B above.

- - -

As the fat-extracting agent, a nonionic type of detergent was chosen because it dissolves by association and can be forced from solution by heat or salt, or both. Triton X-100, an ethylene oxide condensation product, was found to be satisfactory and is specified in the test method, although Tergitol Dispersant-NPX also can be used.

The sodium tetraphosphate is used primarily to disperse the protein and liberate the fat. Additionally, it serves to break the emulsion of detergent and water, and to sequester calcium ions, thus preventing precipitation of calcium salts. Of five polyphosphates that are recognized as distinct compounds, the tetraphosphate was selected as having an optimum combination of properties.

The methyl alcohol decreases the viscosity of the test mixture, so that the fat rises more readily; further, it tends to cause the fat particles to collect into one large globule. The alcohol does not partition into the fat.

Two other important factors in the test are the temperature and time of heating. The temperature of boiling water was selected to obviate the need for thermostatic control, and the detergent and amount of polyphosphate were so chosen that reaction would be effected at this temperature. The heating time, 15 minutes, is considered to be a desirable minimum.

Alternative procedures, with and without centrifuging, are given in Part B of the test. The centrifuge procedure yields fat values which check closely with results obtained in the Babcock test. Results with the non-centrifuge procedure are occasionally slightly low, possibly because of mechanical entrainment of fat particles, and further work is needed to check its accuracy.

The method as given above is designed for milk and cream of less than about 15 percent fat content. For heavier cream the procedure is similar, except that products of 15 to 30 percent FAT content require 10 ml. of reagent A, and still heavier creams, up to about 50 percent fat, require 15 ml. of the reagent.

Discussion

Mr. Scott: Is the BDI test receiving official recognition? Mr. Sager: The AOAC is studying the method. It is not yet officially recognized by the States.

Mr. Heinemann: Considers the heating time -- 15 minutes -- an undesirable

feature of the test. Mr. Sager: The collection of fat is essentially complete at the end of 5 minutes, so that the bath time could be reduced if necessary. The last 10 minutes of heating serves to collect the final 0,1 percent of fat.

Mr. Heinemann: Discussed the relative advantages of BDI and Babcock tests, from the standpoint of time required, particularly in the bath. Dr. Corbett: Centrifuging (and not the bath time) is the main stumbling block of his laboratory in the operation of the Babcock test. Dr. Hartman: The bath time should not be important in scheduled operations involving a large number of samples, using a bath of sufficiently large capacity.

A question was raised as to the effect of milk preservatives. Mr. Sager: Mercuric chloride does not interfere with the test. Chloroform would get into the fat, and make the results too high.

Continuation of Report

The BDI test performs as well with homogenized milk as with plain milk.

The test has various advantages over the Babcock test. The chemicals are not only harmless, but cheap: some of the detergents sold over the counter in grocery stores would probably work as well as Triton X-100. Fewer precautions need be taken as concerns such particulars as temperature of milk and acid, and speed of centrifuge. The centrifuging time is considerably less, and no repeated additions of water are required. Less precision is required of inexperienced operators. At the end of the test, the fat column is transparent, containing no charred particles. The bottom meniscus is uniform, rounded, and clear-cut, not irregular as is often the case in the Babcock test.

The BDI test works well with ice cream. Early in the development of the test, trouble was encountered with a chocolate ice cream which gave no test for fat. The difficulty was traced to the high solids content of the mix, and was remedied by use of an increased quantity of detergent.

Sodium carbonate used in 2-percent solution also has improved the test as applied to ice cream, assisting the polyphosphate in breaking the fat-detergent emulsion. It has general advantages in the test as applied to milk, causing somewhat better fat separation and a somewhat sharper meniscus. Hence we are at the point of making its use a part of the regular procedure.

Recent work has indicated that such compounds as Versene or Sequestrene, which are sodium salts of ethylenediamine tetra-acetic acid, have possibilities as substitutes for polyphosphates as dispersing and sequestering agents. However, further study is necessary to determine the best means of using them.

Mr. Sager distributed a mimeograph circular (BDIM-1154) which included tables comparing fat determinations by the BDI, Babcock, and Roese-Gottlieb (Mojonnier) tests, as applied to milk, ice cream, and evaporated milk. Two of these tables are inserted below.

Table 1. Butterfat in Milk: Comparison of BDI Detergent Test with Babcock Test.

Milk sample[1] (date)	BDI Test[2]	Babcock Test[2]
	Percent	Percent
2-17	4.58	4.57
2-18	4.20	4.15
2-19	4.27	4.20
2-20	4.34	4.29
2-25	4.32	4.29
2-27	4.13	4.10
3-3	3.90	3.80
3-4	4.24	4.19
3-5	3.91	3.90
3-6	4.31	4.30
3-9	3.88	3.91
3-10	4.22	4.30
3-11	4.29	4.27
Average	4.20	4.17

[1] Mixed herd milk from Beltsville Agricultural Research Center.

[2] Both tests were conducted in Babcock test bottles especially selected for accuracy of calibration (tolerance limit 0.02 percent). All values are averages of duplicate determinations. Readings were made from lowest point of fat column to highest point of upper meniscus.

Table 2. Butterfat in Milk: Comparison of BDI Detergent Test with Babcock and Roese-Gottlieb (Mojonnier) Tests.

Milk sample[1] (date)	BDI Test[2]		Babcock Test[2]		Roese-Gottlieb Test[5]
	Official reading[3]	Corrected reading[4]	Official reading[3]	Corrected reading[4]	
3-3	3.90	3.78	3.80	3.65	3.79
3-4	4.24	4.12	4.19	4.04	4.16
3-5	3.91	3.79	3.90	3.75	3.87
3-9	3.88	3.76	3.91	3.76	3.86
3-10	4.22	4.10	4.30	4.15	4.18
3-11	4.29	4.17	4.27	4.12	4.22
4-28	4.20	4.08	4.20	4.05	3.95
5-5	2.88	2.76	2.97	2.82	2.84
5-7	4.22	4.10	4.21	4.06	4.05
5-8	3.72	3.60	3.70	3.55	3.62
5-11	4.24	4.12	4.25	4.10	4.10
5-13	3.81	3.69	3.90	3.75	3.68
5-14	3.90	3.78	3.85	3.70	3.64
5-15	3.72	3.60	3.75	3.60	3.54
Average	3.94	3.82	3.94	3.79	3.82

[1] Mixed herd milk except for one sample of pure Holstein milk.

[2] BDI and Babcock tests were conducted in Babcock test bottles especially selected for accuracy of calibration (tolerance limit 0.02 percent). Values for these tests are averages of duplicate determinations.

[3] From lowest point of fat column to highest point of upper meniscus.

[4] Official reading minus a factor introduced to correct for errors due to depth of upper meniscus. This factor, calculated by Dr. Holm, is 0.12 percent for the BDI test and 0.15 percent for the Babcock test.

[5] Averages of triplicate determinations.

Finally, Mr. Sager presented the following figures, summarizing the most recent data comparing the BDI, Babcock, and Roese-Gottlieb tests.

Sample	Average of	BDI test			Babcock test	Roese-Gottlieb test
		Bottom to bottom	Bottom to top	Bottom to reader		
Milk (mixed-herd)	44		4.41		4.41	
Ice cream (various flavors)	49	12.21		12.31		12.33
Ice cream (vanilla)	26	11.92		12.04		12.064
Ice cream (chocolate)	16	12.66		12.81		12.708
Evaporated milk	3	7.7	8.1			8.043

Mr. Sager, in answer to a question: In the expression "bottom to reader", the "reader" is a mineral oil prepration known as Glymol which is added dropwise to the top of the fat column in the neck of the bottle. The Glymol is not miscible with the fat and eliminates the air-fat interface, thus serving to flatten the meniscus.

Dr. Corbett: Dairy plants buy milk on the basis of the Babcock test, and sell it on the basis of the Mojonnier (Roese-Gottlieb) test. There has existed a need to reconcile the two tests, since discrepancies are the cause of monetary loss. In any comparative studies of the new BDI test, its relation to the Mojonnier test should be emphasized.

Dr. Parfitt: The group here present supervises perhaps 6 million Babcock tests every year. Dr. Corbett's point, that the BDI test should be reconciled with the Mojonnier test, is very pertinent.

Dr. Holm: Discrepancies between the Mojonnier and other tests may be at least partly due to the fact that the ethers used in the Mojonnier test extract phospholipids as well as glycerides, thus tending to give high values.

Mr. Heinemann: Another factor to be considered is that there appears to be a seasonal difference between Babcock and Mojonnier test results.

Dr. Hartman: Would like to see the BDI test tried out in plants, and to have it studied by a committee of the American Dairy Science Association.

Dr. Holm: The AOAC has been interested in making a collaborative study which would compare the BDI and Mojonnier tests, and in which the American Dairy Science Association would doubtless have an important role. As of last year, Mr. Sager was somewhat doubtful that the test was ready for such a study. However, since the discovery that sodium carbonate is a useful adjunct to the polyphosphate, particularly in the case of high-solids samples, Mr. Sager feels that it would be desirable to go ahead with the collaboration.

Dr. Holm, continuing: It would be wise not to look upon the BDI test in its present form as a perfect test. There is still room for improvement. This is not at all discouraging when it is remembered that after 60 years of experience, the Babcock test still has many major faults. If the AOAC finds that the BDI test in its present form is as good as the Babcock test, it will be an important forward step.

Determination of Total Solids of Milk by Means of the Lactometer

Paul D. Watson

Lactometer readings, in combination with the percentage of butterfat, were used by dairy chemists as early as 1841 in efforts to combat the practice of watering milk. However, in those days the methods for the determination of fat were rather crude, and a statement of the quantitative relationship between the fat content, specific gravity, and total solids of milk had to await the development of a quick and reasonably accurate method for determining butterfat, which was achieved by Babcock towards the close of the century. Since that time, the use of formulas for calculating the solids in milk has been common in the United States and abroad, and over three dozen different equations have been proposed by various investigators.

It has often been noted that these formulas appear to give reliable estimates of milk solids only when applied to data from which they were derived. Many researchers have, therefore, substituted constants and corrections in the basic equations in order to make the computed values agree with the experimental ones. The different constants, corrections, and divergencies in the calculated values for total solids have caused confusion, and have cast doubt upon the fundamental reliability of the procedure.

To a large extent, the lag in the development of a truly satisfactory method for milk solids is attributable to the emphasis that for many years has been placed upon the butterfat content of milk as a basis for payment to farmers. However, the increasing importance of the nonfat solids in the economy of the dairy industry has made it imperative to have a method for their determination which would be comparable in speed and accuracy to the Babcock fat test. Hence the Dairy Products Section has devoted considerable effort to an analysis of the problem, and to the development of a convenient and practical method which would eliminate or minimize the difficulties previously encountered.

Sources of Error in Previous Work

A study of the literature indicated that the unsatisfactory results obtained by previous workers were due largely to two factors. One of these is the indefinite physical state of milk fat at 60°F., the temperature generally used for lactometer measurements. The uncertain degree of solidification of the fat at this temperature makes it unlikely that consistent values will be obtained. The need for having the fat in a more uniform state has been recognized for many years, but progress toward a solution to the problem has been slow. Various workers have preheated the milk to 104°F., or even as high as 113°F.; but before making the lactometer readings, they have cooled it again to 60° or thereabouts.

The second factor causing trouble in the determination of total solids is the general use of poorly-designed and inaccurately-calibrated lactometers by dairy scientists and technologists. ost lactometers in use today appear not to have been checked for accuracy, or to have been checked only in some unsatisfactory way; certainly, none appear to have been certified by the National Bureau of Standards. The literature indicates that it is common practice to calibrate lactometers in solutions of salts, acid, or sugar, which have a surface tension considerably higher than that of milk; invariably, no mention is made of any correction for the differences in surface tension and the meniscus, the neglect of which can cause appreciable errors in the evaluation of the various formulas for calculating milk solids. Some workers have tried to avoid errors by calibrating their lactometers in milk. The use of whole milk in pycnometers is impractical, due to the rising of the fat and also because of variations in the physical state of the fat at 60°F.; and if skim milk is used, it is possible to check the scale at only one point, whereas three check points are desirable.

A survey of commercial lactometers indicates that of a half-dozen types available, none is entirely suitable for accurate milk-solids determinations. The sturdy, practical types are so coarsely graduated that readings of the required precision cannot be obtained, while the finely-graduated instruments are far too fragile for general use. And all types examined have been found to give inaccurate readings when compared to lactometers calibrated by the National Bureau of Standards.

In connection with the precision of readings, some workers have held that in determining total solids, it is unnecessary to read the lactometer closer than 0.5 of a degree, since this value is comparable to an error of only 0.1 percent in the Babcock butterfat determination. However, in determinations of solids-not-fat, a variation of 0.5 lactometer degree will result in an error of about 0.14 percent in solids-not-fat, and it is hence desirable that the lactometer be readable to 0.2 degree.

Proposed Modifications of the Lactometer Procedure

Temperature of measurement. Consideration of the temperature problem has led to the conclusion that the lactometer method can be made more practical by eliminating the "cooling down" step which has been generally recommended in the past. It is proposed to heat the milk sample to a temperature at which milk fat is in a uniform liquid state, and read the lactometer at this temperature. Since the milk samples are held for several minutes while a reading is

made, it is desirable that the temperature be just high enough to insure fat liquidity, but not so high that other physical changes would occur to affect the specific gravity of the milk. Experiments showed that consistent, reproducible results can be obtained if the holding temperature is 102°F. (39°C.), which is slightly above the body tempe ature of the cow. This figure is consistent with the literature on the melting range of butterfat.

No provision is made for temperature variations and consequent corrections, since 102°F. is a minimum temperature, and corrections could be a source of unnecessary error. The temperature can be readily maintained by use of a water bath equipped with thermostatic control.

Milk samples may be rapidly heated to near the proper temperature by brief immersion in hot water before transferring to lactometer cylinders in the bath, where they are held at 102°F. for about 3 minutes before the lactometer is read. Before using, lactometers should be prewarmed in the water bath for a few minutes and dried with a cloth. It has been found that the samples of milk may be held in the bath for an hour or more at the specified temperature without any appreciable change in the lactometer readin s, provided the samples are stirred briefly before the reading. This makes it possible to carry out, in a time-saving manner, successive readings on a considerable number of samples while they are all immersed in the bath, with the certainty that temperature equilibrium has been attained.

Lactometer design. Concurrently with the matter of sample temperature, attention has been given to lactometer design. In the preliminary experimental work on milk solids, the Dairy Products Section had used a fragile type of commercial lactometer, graduated to read to 0.1 of a lactometer degree at 60°F., and checked by the National Bureau of Standards. When this was used at 102°F., the total-solids formula included an undesirably large correction factor for the difference in expansion of glass and milk over the ra ge 60 to 102°. Consequently, the Section designed a new glass lactometer which is calibrated to read the specific gravity of milk at 102°F., referred to water at 102°F., over the range between 26 and 37 lactometer degrees. It was necessary to provide a rather large bulb in order to secure an instrument with both a sturdy stem and sufficient sensitivity to read to 0.2 degree (two in the fourth decimal place, expressed as specific gravity). A milk sample of about 10 ounces is required. This lactometer is now on the market.

Since the nature of glass somewhat limits the durability and sensitivity desirable in a lactometer of reasonably small size, the Section has developed an aluminum model which has very interesting possibilities from the standpoints of sensitivity, ease of reading, and unbreakability.

Results with Modified Lactometer Procedure

Mr. Watson distributed a circular (BDIM-1155) containing the results of more than 80 milk solids determinations by lactometer readings at 102°F., as compared with results by the Mojonnier vacuum-oven method. The samples included milk from individual cows, small groups of cows, and mixed herds, as well as skim milk and milk containing added water or skim-milk solids. Each determination was run in duplicate.

For 63 samples, lactometer readings were made with a precision instrument calibrated by the Bureau of Standards for use at 60°F. The following formula was developed to cover its use at 102°:

$$TS = 1.262\ F + \frac{273\ (L + 6)}{L + 1000},$$

where TS = total solids (percent), F = fat (percent), and L = lactometer reading (degrees).

For 21 samples, measurements were made with the new glass lactometer designed by the Dairy Products Section specifically for use at 102°. Here the formula used was:

$$TS = 1.262\ F + \frac{273\ L}{L + 1000}.$$

In all cases, the fat determination was made by the Babcock method, with accurately-calibrated glassware. In many cases, the Babcock results were checked by gravimetric methods.

Very good agreement was obtained between lactometer and vacuum-oven methods. For the 63 samples in which the precision lactometer was used, the mean variation was less than 0.003 percent total solids. For the 21 samples in which the new Dairy Products lactometer was used, the mean variation was 0.010 percent. For individual samples, the variation was generally less than 0.10 percent, and only infrequently did it exceed 0.20 percent.

Mr. Watson observed that when fat is determined by the BDI detergent test instead of by the Babcock test, the results with the Dairy Products lactometer are fitted into a slightly different formula:

$$TS = 1.33\ F + \frac{273\ L}{L + 1000} - 0.3$$

This preliminary work indicates that the modified lactometer method gives results which should compare favorably in accuracy with any other method, and should be practical for general use. The method is being further checked in cooperative studies at the University of Maryland and the University of Wisconsin, sponsored by the American Dairy Association.

Discussion

Dr. Parfitt: Have you used the new lactometer method in studies of milk adulteration, such as the New York City Board of Health has conducted? Mr. Watson: No; however, it might be useful in this connection.

Mr. Heinemann: The lactometer test is influenced by abnormalities in the protein and lactose content of milk. Mr. Whittier: The calculated protein content of the total milk solids is approximately constant at 26 1/2 percent, regardless of the fat concentration. However, the lactose content is in inverse

relation to the amount of fat. The specific gravity of nonfat milk solids is 1.61 - .0064•Fat.

Dr. Holm: A difficulty in lactometer work has been the assumption that the specific gravity of nonfat solids does not change. This assumption is incorrect. The formula corrects for the change in the density of the solids. Dr. Parfitt: This consideration is important in the evaporated milk industry. In some areas, there is a variation in yield of as much as one pound of milk in a case.

Dr. Holm: Several years ago we determined the yield of cheese from milk produced at different times of the year. There was a sharp drop in the yield curve in May, when cows are put out on pasture. Mr. Heinemann: In this connection, the determination of total solids with a lactometer works well in any month except May.

--o--